American Fern Journal 95(4):153–159 (2005)

The Young Gametophyte of *Lycopodiella lateralis* and the Role of the Intermediate Shaft in Development of *Lycopodiella* Gametophytes

DEAN P. WHITTIER
Department of Biological Sciences, Box 1634, Vanderbilt University, Nashville, TN 37235-1634
KAREN SUE RENZAGLIA
Department of Plant Biology, Southern Illinois University, Carbondale, IL 62901-6509

ABSTRACT.—Spores of *Lycopodiella lateralis* germinate rapidly in illuminated cultures as is typical for most *Lycopodiella* spores. The young gametophyte develops into a solid, green, spherical primary tubercle. Mature gametophyte development occurs at the top of the primary tubercle by the formation of a crown with photosynthetic lobes. An intermediate shaft reported for other *Lycopodiella* gametophytes fails to form. Comparisons were made between intermediate shafts of *Lycopodiella* gametophytes growing in nature and in culture. In well illuminated conditions the intermediate shaft does not form as it does on poorly illuminated gametophytes. If the gametophyte development starts partially covered by soil, long intermediate shafts can be produced. The intermediate shaft raises the top of the young gametophyte to the top of the soil where a sexually-mature gametophyte develops and sexual reproduction can take place. The intermediate shaft provides the possibility for a young gametophyte in unsuitable illumination to grow into more suitable illumination for gametophyte maturation.

The spores of less than 5% of the species in the Lycopodiaceae have been germinated. Germination under natural conditions suggests that spores of the Lycopodiaceae fall into two classes according to how fast they germinate (Bierhorst, 1971). Spores of species with mycorrhizal gametophytes germinate slowly and those of species with photosynthetic gametophytes (*Lycopodiella*; after Øllgaard, 1987, 1989) germinate rapidly. The observations on the rapid germination of *Lycopodiella* spores (DeBary, 1858; Treub, 1884, 1887, 1888) are based on four species – *Lycopodium inundatum* L. (*Lycopodiella inundata* (L.) Holub), *Lycopodium cernuum* L. (*Lycopodiella cernua* (L.) Pic. Serm.), *Lycopodium salakense* Treub (*Lycopodiella*) and *Lycopodium curvatum* Sw. (*Lycopodiella*).

Information on early development of *Lycopodiella* gametophytes is primarily based on the above mentioned species. The development of the shape and size of the primary tubercle was described by DeBary (1858) and Treub (1884, 1887, 1888). Subsequent gametophyte development was reported for three of these species by Treub (1884, 1888) and Goebel (1887). More information is available on the structure of mature gametophytes of *Lycopodiella* than on immature gametophytes (Bruce 1979). This is true for the gametophytes of *Lycopodiella lateralis* (R. Br.) B. Øllg. Mature gametophytes of that species were described by Holloway (1916, 1920) and Chamberlain (1917) as having characteristics typical of *Lycopodiella* gametophytes. Mature gametophytes of *L. lateralis* have also been grown in axenic culture for a study on the fine structure of its

spermatozoid (Maden *et al.*, 1997). No information was reported on the earliest stages of gametophyte development in any of these studies.

In general treatises the gametophyte of *Lycopodiella* is often described as having an upright green cylindrical body bearing numerous green lobes at its top (Bower, 1908; Campbell, 1928; Eames, 1936). More detailed studies on these gametophytes have demonstrated a more complicated structure. Treub (1884) first described the gametophyte of *Lycopodiella cernua* with three regions – a basal primary tubercle, a middle cylindrical portion, and a crown with lobes. Holloway (1916) used the terms – primary tubercle, intermediate shaft, and crown of lobes in his studies on *Lycopodiella* gametophytes and these terms will be followed in this report.

Differences in structure are known to exist among *Lycopodiella* gametophytes. Variations in the primary tubercle, transition to mature gametophyte, and type of photosynthetic lobes have been reported. Also, some species have spores that germinate slowly in axenic culture as opposed to the rapid spore germination of other species under natural conditions (Whittier, 1998). This study was undertaken in an effort to provide additional information on the speed of spore germination and early gametophyte development for *Lycopodiella* and more specifically *L. lateralis*.

Materials and Methods

Plants of *Lycopodiella lateralis* (R. Br.) B. Øllg. collected in New South Wales, Australia (*Renzaglia #932*) were the source of the spores for this study. The spores were pre-wet for one day and then they were surface sterilized with 20% Clorox (1.1% sodium hypochlorite) by the method of Whittier (1964), suspended in sterile water, and sown on 14 ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were tightened after inoculation. Young gametophytes were moved from the culture tubes to Petri plates with 50 ml of nutrient medium for further growth. Most of the cultures were maintained under a 14 hour photoperiod (50 $\mu m \cdot m^{-2} \cdot sec^{-1}$) under Gro-lux fluorescent lamps at 22±1°C. A few culture tubes were placed in the dark immediately after their inoculation as a control.

The nutrient medium contained 100 mg NH_4NO_3, 50 mg $MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2$, and 50 mg K_2HPO_4 as a final concentration per liter. In addition 4 ml of a FeEDTA solution (Sheat *et al.*, 1959) and 0.25 ml of a minor element solution (Whittier and Steeves, 1960) were added per liter. The nutrient medium was adjusted to pH 5.8 before autoclaving and it was solidified with 1.1% agar.

The percentage of germination was determined by examining 500 spores for each observation. Some young gametophytes were cleared and their nuclei stained with an acetocarmine-chloral hydrate treatment (Edwards and Miller, 1972).

Results

Spore germination for *L. lateralis* was rapid in illuminated cultures. Although no germination had occurred 12 days after sowing, 1% of the

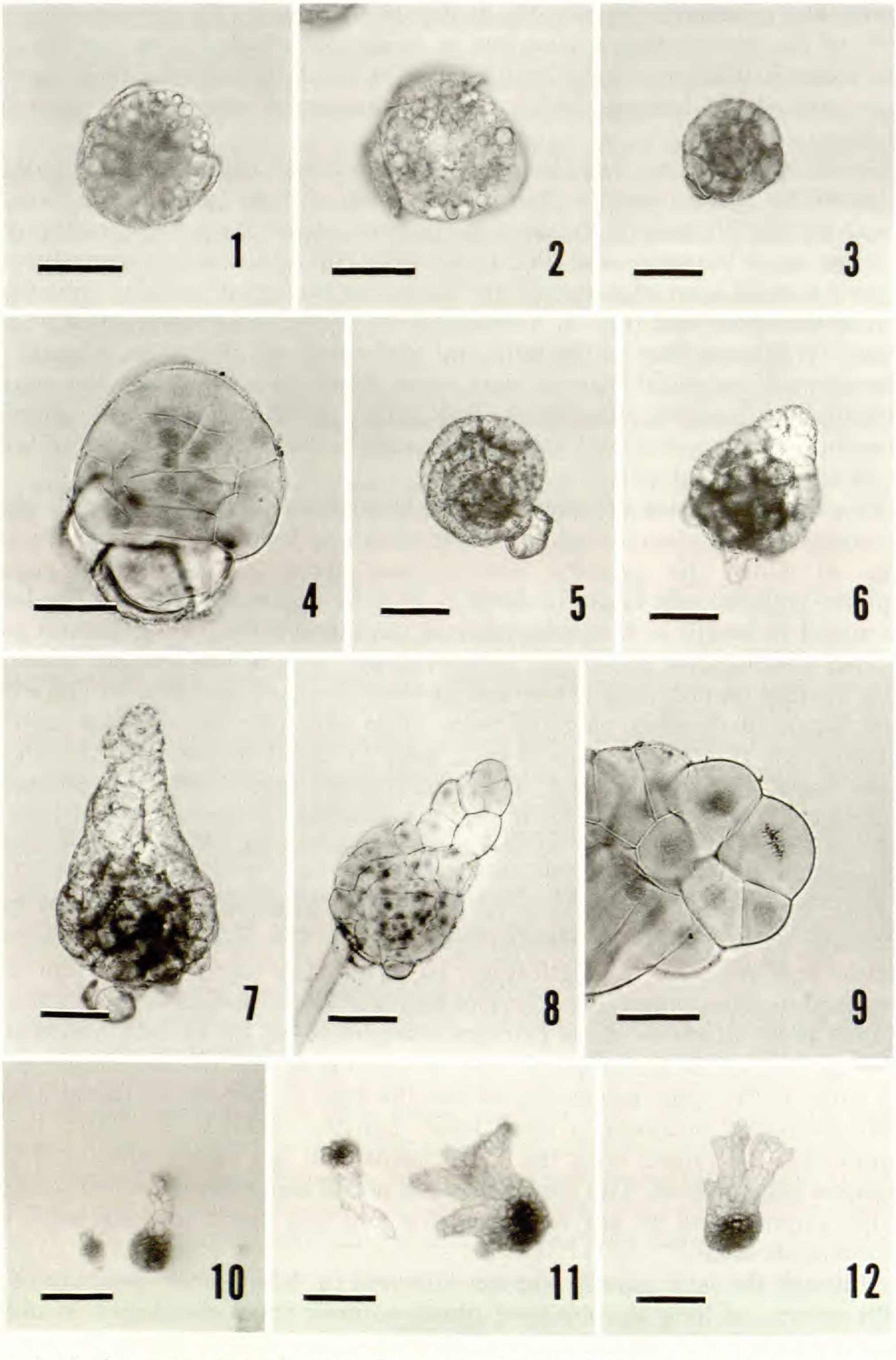
spores had germinated by day 13. At day 16 there was 14% germination and 88% of the spores were giving rise to young gametophytes by day 35. No spore germination occurred after 6 months in the dark; however, these spores were still viable because 54% of them germinated when illuminated for 21 days.

Germination occurred as the spore expanded and ruptured the triradiate ridge of the spore coat; the first division was oblique to the polar axis of the spore (not illustrated). Further enlargement caused the young gametophyte to bulge out of the spore coat (Fig. 1). Irregular cell divisions in various planes formed a small mass of gametophyte tissue that remained partially contained within the spore coat (Fig. 2). Gametophytes at this stage were usually light green. With more time in the light and additional cell divisions, a small, 3-dimensional, spherical mass of dark green tissue developed and the young gametophyte escaped the spore coat (Figs. 3, 4). Cleared and stained gametophytes demonstrated their solid nature by revealing the nuclei of both surface and internal cells (Fig. 4).

The spherical mass of green gametophyte tissue increased in size with additional cell divisions and cell enlargement to form the primary tubercle (Fig. 5). Once the primary tubercle was about 200 μm in diameter, a photosynthetic lobe began to develop from its upper end (Fig. 6). The lobe increased in length as it developed from the tubercle (Fig. 7). A cleared and stained gametophyte shows the 3-dimensional mass of the primary tubercle with the thin photosynthetic lobe arising from its top surface (Fig. 8). Once the lobe began to develop, rhizoids were often evident from the base of the gametophyte (Figs. 6, 8). Apical cells were involved in the early growth of these lobes (Figs. 8, 9) and, occasionally, an apical cell was observed undergoing cell division (Fig. 9). No filamentous outgrowths formed from the tubercles or lobes and no secondary tubercles developed under these conditions.

With more growth, the base of the young gametophytes enlarged but remained more or less spherical (Figs. 10, 11). The initial lobes remained narrow and increased in length (Figs. 10, 11) or they increased in width and branched to some extent (Fig. 12). The length of these lobes rarely was 3 times as long as the diameter of the primary tubercles (Figs. 10, 11, 12). Additional lobes formed as the gametophyte base enlarged (Fig. 11). The development of the latter on the larger gametophytes was the same as that for the initial lobes. More branching occurred in these lobes than the initial lobes. Under these conditions, the crown with the lobes formed on the upper surface of the enlarged gametophyte. The crown was not raised above the spherical portion of the gametophyte by any tissue or structure that could be considered an intermediate shaft.

Although the later growth was not followed in detail, larger gametophytes with crowns of long strap-shaped photosynthetic lobes developed in older cultures. Mature gametophytes developed and the moisture on the surface of the nutrient medium was sufficient to allow fertilization and the formation of sporophytes in four months.



FIGS. 1–12. Immature gametophytes of *Lycopodiella lateralis*. 1. Germinating spore with bulging cell. 2. Young gametophyte contained by spore coat. 3. Small spherical gametophyte. 4. Small

Discussion and Conclusions

Spores of *Lycopodiella lateralis* began to germinate in less than two weeks and almost 90% of them had germinated by the 5th week. This rate of germination compares favorably with the rate reported for *L. inundata*, *L. cernua*, *L. curvatum*, and *L. salakense* (DeBary, 1858; Treub, 1884, 1887, 1888; Whittier, 1998). The rate of spore germination in *L. lateralis* is much faster than those reported for the spores of species of the Lycopodiaceae that form non-photosynthetic, mycorrhizal gametophytes. The adage that *Lycopodiella* spores germinate rapidly holds true for *L. lateralis*.

Early development of gametophytes of *L. lateralis* is similar to that of *L. inundata*, *L. cernua*, *L. salakense*, and *L. curvatum* (DeBary, 1858; Treub, 1884, 1887, 1888). In all cases a small, solid, globular body of cells, the primary tubercle, forms. It has an oblong shape for *L. inundata*, *L. cernua*, and *L. salakense* (DeBary, 1858; Treub, 1884, 1888). The present study shows the spherical shape of the primary tubercle for *L. lateralis*. The basal region of the older tubercle is pointed in *L. inundata* and *L. carolinianum* (Goebel, 1887; Bruce, 1979) and rounded in *L. cernua* (Treub, 1884). The spherical primary tubercle of *L. lateralis* from culture provides the older tubercle with a rounded base as reported by Holloway (1916) from natural conditions.

Many *Lycopodiella* gametophytes under natural conditions are not completely green. It has been noted that the bases of some have little chlorophyll and are not green (Bruce, 1979). Holloway (1916) reported that only the photosynthetic lobes of *L. lateralis* from soil were green, however Chamberlain (1917) did not report non-green portions of these gametophytes. The gametophytes of *L. lateralis* in culture are dark green from the early stages of primary tubercle formation to mature gametophyte. There are no pale green or colorless regions. Cultured gametophytes are completely illuminated on agar and there is no shading. It would appear that the more or less colorless regions of *Lycopodiella* gametophytes in nature are those portions sunken in or shaded by the soil, where chlorophyll production would be inhibited.

The photosynthetic lobes on the crown of *Lycopodiella* gametophytes also exhibit variation. They have been variously described as rounded (Treub, 1884; Goebel, 1887), leaf-like (Bower, 1908; Campbell, 1928) or rudimentary (Treub, 1887). Mature gametophytes of *L. lateralis* from nature have been described as having leafy (Chamberlain, 1917) or filamentous lobes (Holloway, 1916). The young gametophytes of this species from culture have narrow

←

---

spherical gametophyte with spore coat; cleared and stained to show nuclei. 5. Primary tubercle with spore coat. 6. Primary tubercle with young rhizoids and early stage of photosynthetic lobe development. 7. Gametophyte with older stage of lobe formation. 8. Gametophyte with rhizoid; cleared and stained to show nuclei of tubercle and young lobe. 9. Apex of cleared and stained lobe with a metaphase plate in apical cell. 10. Gametophyte with narrow lobe. 11. Older gametophyte with several developing lobes and younger gametophyte with single narrow lobe. 12. Gametophyte with branched lobe. Scale bars in Figs. 1, 2, 4 and 9 = 50 μm; Figs. 3 and 5–8 = 100 μm; Figs. 10–12 = 500 μm.

pointed lobes. On more mature gametophytes, the photosynthetic lobes are strap shaped. It is not understood at this time why variation occurs in the photosynthetic lobes of *L. lateralis*.

Treub (1884) described in *L. cernua* the formation of an elongated structure from the top of the primary tubercle that he called the cylindrical portion (intermediate shaft as identified by Holloway (1916) for *L. lateralis*). From the top of the intermediate shaft, the crown with photosynthetic lobes is formed. The intermediate shaft is a variable element in the morphology of *Lycopodiella* gametophytes. Goebel (1887) illustrated short intermediate shafts in the gametophytes of *L. inundata* and Treub (1888) found that gametophytes of *L. salakense* had long narrow, almost filamentous, intermediate shafts. In a more recent study Bruce (1979) demonstrated gametophytes of *L. carolinianum* with long intermediate shafts between the gametophyte base and the crown with photosynthetic lobes. Variation in this structure has been reported for *L. lateralis* in nature. Chamberlain (1917) described short stout gametophytes basically without intermediate shafts. However, in addition to this type of gametophyte Holloway (1916, 1920) described others with drawn out intermediate shafts.

The length of the intermediate shaft appears to be controlled by light. Bruce (1979) suggested that the length of the shaft between the base and mature region of gametophytes of *L. carolinianum* depended on how deep in the soil spore germination occurred. Gametophytes of *L. carolinianum* grown in illuminated axenic cultures lacked any elongated regions (unpubl. data). The intermediate shaft described for some gametophytes of *L. lateralis* is absent from gametophytes grown in culture. Well illuminated conditions eliminate the intermediate shafts of gametophytes of *L. carolinianum* and *L. lateralis* as described from nature.

The plasticity of the intermediate shaft in *Lycopodiella* is important for gametophyte development and success. Its role in gametophyte development insures that the top of the gametophyte is well illuminated. If a young gametophyte develops in a well illuminated site, the intermediate shaft does not form. If a young gametophyte develops in a poorly illuminated site, the intermediate shaft grows until its apical region is better illuminated. With adequate illumination the top of the intermediate shaft can initiate the development of the crown and photosynthetic lobes of the mature gametophyte.

Because there is the possibility that spores may fall into less than favorable sites, variability in development provides a mechanism to increase the chance for these spores to give rise to mature gametophytes. Development of the intermediate shaft provides the opportunity for a gametophyte initiated deeper in the soil to reach the soil surface where a photosynthetic, sexually-mature gametophyte can form and sexual reproduction can occur.

ACKNOWLEDGMENTS

This study was supported by the NSF, ATOL Program, Green Tree of Life Project to KSR and by the Vanderbilt University Research Council to DPW.

Literature Cited

Bierhorst, D. W. 1971. *Morphology of vascular plants.* Macmillan, New York.

Bower, F. O. 1908. *The origin of a land flora.* Macmillan, London.

Bruce, J. G. 1979. Gametophyte and sporophyte of *Lycopodium carolinianum.* Amer. J. Bot. 66:1156–1163.

Campbell, D. H. 1928. *The structure and development of mosses and ferns.* 3rd ed. Macmillan, New York.

Chamberlain, C. J. 1917. Prothallia and sporelings of three New Zealand species of *Lycopodium.* Bot. Gaz. (Crawfordsville) 63:51–65.

Debary, A. 1858. Sur la germination de Lycopodées. Ann. Sci. Nat. Bot. ser IV, 9:30–36.

Eames, A. J. 1936. *Morphology of vascular plants, lower groups.* McGraw-Hill, New York.

Edwards, M. E. and J. H. Miller. 1972. Growth regulation by ethylene in fern gametophytes. III. Inhibition of spore germination. Amer. J. Bot. 59:458–465.

Goebel, K. 1887. Ueber Prothallien und Keimpflanzen von *Lycopodium inundatum.* Bot. Zeit. 45:161–168,177–190.

Holloway, J. E. 1916. Studies in the New Zealand species of the genus *Lycopodium.* Part I. Trans. New Zealand Inst. 48:253–303.

Holloway, J. E. 1920. Studies in the New Zealand species of the genus *Lycopodium.* Part IV-the structure of the prothallus in five species. Trans. New Zealand Inst. 52:193–239.

Maden, A. R., D. P. Whittier, D. J. Garbary and K. S. Renzaglia. 1997. Ultrastructure of the spermatozoid of *Lycopodiella lateralis* (Lycopodiaceae). Canad. J. Bot. 75:1728–1738.

Øllgaard, B. 1987. A revised classification of the Lycopodiaceae *s. lat.* Opera Bot. 92:153–178.

Øllgaard, B. 1989. Index of the Lycopodiaceae. Biol. Skr. 34:1–135.

Sheat, D. E. G., B. H. Fletcher and H. E. Street. 1959. Studies on the growth of excised roots. VIII. The growth of excised tomato roots supplied with various sources of nitrogen. New Phytologist 58:128–154.

Treub, M. 1884. Études sur les Lycopodiacées. I. Le prothalle du *Lycopodium cernuum* L. Ann. Jard. Bot. Buitenzorg 7:107–138.

Treub, M. 1887. Some words on the life-history of lycopods. Ann. Bot. 1:119–123.

Treub, M. 1888. Études sur les Lycopodiacées. IV. Le prothalle du *Lycopodium salakense.* Ann. Jard. Bot. Buitenzorg 7:141–146.

Whittier, D. P. 1964. The effect of sucrose on apogamy in *Cyrtomium falcatum.* Amer. Fern J. 54:20–25.

Whittier, D. P. 1998. Germination of spores of the Lycopodiaceae in axenic culture. Amer. Fern J. 88:106–113.

Whittier, D. P. and T. A. Steeves. 1960. The induction of apogamy in the bracken fern. Canad. J. Bot. 38:925–930.